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Enhancement of Astaxanthin Biosynthesis

It has also been found that when antimycin or another inhibitor of the main respiratory chain are added to Phaffia rhodozyma cells, and the cells are exposed to light, the astaxanthin content of the yeast is considerably enhanced. The underlying mechanism for this phenomena is not understood, but it could be hypothesized that when the primary respiratory pathway is inhibited, light acts as one of the triggers of a secondary respiratory (oxidative) pathway, having a net effect of considerably stimulating the production of astaxanthin. Thus, the present invention comprises processes for increasing the astaxanthin or other carotenoid content of yeast, comprising growing the yeast in the presence of a metabolic pathway inhibitor while inducing a secondary respiratory pathway. The secondary respiratory pathway may be induced by such influences as light, certain environmental conditions such as those known to cause stress, nutrients, etc. The present invention is, however, not limited by the above hypothesis.

It will be seen from the experiments discussed below that enhanced astaxanthin biosynthesis can be induced by the above mentioned combination of respiratory chain inhibitor with initiation of the secondary respiratory channel.

Example 9

P. rhodozyma strains used were the natural isolate UCD-FST-67-385 (Phaff et al., 1972; Miller et al., 1976), mutant Ant-1-4 used above, and strain 18-13-6, an astaxanthin enhanced mutant obtained by ethylmethane sulfonate (EMS) mutagenesis procedures (isolated on YM agar). They were grown in yeast extract/malt extract/peptone/glucose medium (YM medium, Difco Co., Detroit, MI) as previously described (An et al., 1989) in a temperature controlled incubator/shaker (Environ-Shaker Model 3597, Lab-Line Instruments, Inc., Melrose Park, IL). Growth was determined by the optical density (660 nm) of a washed cell suspension; 1 mg dry cell weight per ml corresponds to an O.D. of 1.35.

Light was supplied by two Sylvania 20 watt Coolwhite fluorescent tubes held 20-40 cm from the flask media surfaces. ~~Agar media (10 cm diameter)~~ Flasks were inoculated with approximately 10^2-10^6 cells of actively growing yeast. For dark controls, flasks were wrapped in aluminum foil. When insoluble chemicals were included in the media, they were first dissolved in a small quantity of ethanol (0.3% final conc.), which did not affect yeast growth or pigmentation.

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Carotenoid Extraction and Analysis

P. rhodozyma was grown for 5 days in flasks before extraction. Yeasts were harvested from liquid media by centrifugation. The yeast cells were suspended in distilled water, washed in water, and extracted and analyzed for carotenoids by thin-layer chromatography and absorption spectroscopy as previously described (An et al., 1989).

Secondary Respiratory Pathway Induction Increases Carotenoid Production

The influence of two 20 watt fluorescent bulbs placed 20 cm from the surface of P. rhodozyma natural isolate, UCD-FST-67-385, and its antimycin-sensitive mutants, Ant 1-4 and 18-13-6 was studied.

Separate cultures were grown under conditions of (a) total darkness for 30 hours (hereafter "dark"), (b) total darkness with 0.2 μM antimycin (antimycin being introduced at inoculation) (hereafter "dark/antimycin"), and under conditions (c) and (d) which were identical to (a) and (b) except for exposure of the cultures to light from approximately 30 hours into the experiment until approximately 60 hours into the experiment (hereafter "light" and "light/antimycin").

Extraction of the pigments and characterization indicated that the pigments were qualitatively similar in composition, except that more cis-astaxanthin and lower carotene concentrations were found to be present in light grown cells.

Analysis of yeasts showed that the carotenoid content of light/antimycin P. rhodozyma UCD-FST-67-385 increased by two-fold over any of the dark, dark/antimycin or light cultures of this natural isolate (Table 1). Table 2 shows that mutant 18-13-6, which is antimycin sensitive, produced two-fold increase in carotenoid content in antimycin/light over the light culture.

Table 1

Conditions	Growth (mg/ml)	Carotenoid (µg/g y)
Dark	4.4	520
Dark + Ant	1.6	480
Light	3.3	440
Light + Ant	2.2	1000

Table 2

Antimycin	Not added	Added
Light	Carotenoid Content (µg carotenoids/g yeast)	
White	540	1410
Blue	1050	1360
Red	960	1210
Dark	830	1013

As can be seen from the above results, growth and carotenoid formation of natural and astaxanthin enhanced P. rhodozyma is clearly influenced by light. The natural isolate, UCD-FST-67-385, and its antimycin-sensitive mutants ant-1-4 and 18-13-6, each grew better and had increased pigmentation in the dark, but were differently affected by light. --